ISOLATION OF RNA FROM PITH OF METROXYLON SAGO

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LIST OF ABBREVIATIONS

AGE	Agarose Gel Electrophoresis
cDNA	Complementary Deoxyribonucleic Acid
CIA	Chloroform isoamyl
CTAB	Cetyltrimethylammonium bromide
DEPC	Diethylpyrocarbonate
DNA	Deoxyribonucleic
DNase	Deoxyribonuclease
DSP	Dalat Sago Plantation
EDTA	Ethylenediamineetetraacetic
ETBR	Ethidium Bromide
LiCl	Lithium Chloride
MSP	Mukah Sago Plantation
PVP	Polyvinylpyrrolidone
RNA	Ribonuclease
RNase	Ribonuclease
rpm	Revolution Per Minute
SDS	Sodium Dodecyl Sulfate
SSP	Sebakong Sago Plantation
TE	Tris- EDTA

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Isolation of RNA from Pith of *Metroxylon sago*

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ABTRACT

Metroxylon sagu is a type of starch crop that has a great potential to become a valuable commercial plant. *M. sagu* is also known locally as sago palm. Genetic engineering studies such functional genomics expression require RNA as their starting material. However, to isolate RNA is slightly difficult especially for plant that contain high polysaccharides, for example, in sago palm. A specific and effective protocol is needed to obtained high quality of RNA for many gene expression studies Thus this study was conducted to isolate RNA from the pith of sago palm and to study the effectiveness of the protocols. The described protocols are listed as CTAB-LiCl Method I, CTAB-LiCl Method II, CTAB-LiCl Method II and CTAB-Isopropanol. CTAB-LiCl Method III has yield the highest of RNA of 0.025 to 0.144 μg/μl. At $A_{260/280}$ the ratio was ranged from 1.521 to 1.651 while at $A_{260/230}$ the ratio ranged from 0.534 to 1.550. The result indicates that using CTAB-LiCl Method III was successfully isolate RNA from sago pith, however slightly contaminated with protein and organic compound.

Keywords: *Metroxylon sago*, CTAB-LiCl Method, CTAB-Isopropanol Method, total RNA isolation.

ABSTRAK

Metroxylon sagu merupakan tumbuhan kanji yang mempunyai potensi yang tinggi untuk dijadikan sebagai tanaman kormersial. M. sagu turut dikenali sebagai pokok sagu dalam kalangan penduduk tempatan. Kejuruteraan genetik seperti kajian pengekspressan fungsi genomik memerlukan RNA sebagai bahan permulaan kajian. Walaubagaimanapun, pemencilan RNA adalah sukar berikutan kandungan polisakarida yang tinggi dalam pokok sagu. Satu kaedah yang spesifik dan berkesan adalah diperlukan bagi membolehkan RNA berkualiti tinggi berjaya dipencilkan bagi kegunaan kajian genetik ekspressi. Kajian ini memfokuskan kepada pemencilan RNA daripada empulur pokok sagu dan mengkaji keberkesanan kaedah yang sedia ada. Berikut merupakan kaedah yang digunakan dalam kajian ini, CTAB-LiCl kaedah I, CTAB-LiCl kaedah II, CTAB-LiCl kaedah III dan CTAB-Isopropanol. CTAB-LiCl kaedah III adalah kaedah yang telah memencil RNA sebanyak 0.025 to 0.144 μg/μl. Purata pada ada $A_{260/280}$ adalah 1.521 to 1.651 manakala purata pada $A_{260/230}$ adalah 0.534 to 1.550. Keputusan menunjukkan bahawa dengan menggunakan CTAB-LiCl kaedah III telah berjaya memencilkan RNA namun begitu RNA yang diperolehi sedikit tercemar dengan protein dan sebatian organik.

***Kata Kunci**: Metroxylon sago, Kaedah CTAB-LiCl, Kaedah CTBA-Isopropanol, Pemencilan RNA*

1.0 INTRODUCTION

Mextroxylon sagu or locally known as sago palm, mostly grow at the peat swamp as it can tolerate with the environment although the surrounding was not favorable for certain tropical crops to grow. There are many places where *M.sagu* can be found around the world including Thailand, Peninsular Malaysia and Indonesia. (McClatchey *et al.*, 2006). In Sarawak, mostly *M.sagu* can be found in area of Mukah, Dalat, Igan and Daro (M. Hasnain, 2011). There are also sago plantations established in Sarawak for example Mukah Sago Plantation (MSP), Sebakong Sago Plantation (SSP) and Dalat Sago Plantation (DSP) (Noraini *et al.*, 2005). All of them were mainly grown on deep peat while the wild type sago palms were commonly grown in the tropical lowland forest and freshwater swamps which usually can be found near sea level.

Sago palms are well known for its function in production of starch as according to Jong (1995) sago palm is consider as 'starch crop of the 21st century' by many scientist. Sago starch accumulates in the pith core of the stem of the sago palm (Cecil *et al.*, 1982). Trunk formation starts during the third and fourth year growth of the palm (Kueh, 1977). The vegetative phase in the sago palm lasts 7–15 years during which time, the pith is saturated with starch from the base of the stem upwards (Kraalingen, 1986). The sago palm produces vertical trunk that may reach 7–15 m in length and achieve an average thickness of 120 cm at the base of the palm (Flach & Schuiling, 1989). Most of the starch is found in the pith of the palm. The pith typically contains about 250 kg of starch, 425 kg of water and 175 kg of other material (Flach, 1983). As for total collection of starch can reach up to 25- ton of starch from sago plantation under development in the Malaysian state of Sarawak (Ishizaki, 1997).

There are two primary uses of the sago palm one is for the production of edible starch and another one is a durable leaf thatch. Other examples of sago palm products are such bio-fuel, as derivative in paper industry and even as animal feed. Nowadays more and more sago palm is made for animal feed, for example the sago palm waste '*hampas*' which still consists starch within it can be use for foodstuff for the swine as proven by Awg Adeni *et al* (2010). Besides, according to Stanton (1972) the research of sago palm has started to arise due to the increase of economic value of sago palm but yet still lots knowledge about sago palm still need to be addressed and discovered.

In this study, total RNA is isolated from bottom part of pith of sago palm because it is an important step in genetic expression studies such as functional genomics expression. The RNA later can be used to construct cDNA library as cDNA later will be use to generate template for screening for genes which might be useful in improvement in sago palm.

Sago palm is a kind of plant that contains high polysaccharides and secondary metabolites compounds in its tissue thus obstruct the successful of RNA extraction. Polysaccharide and secondary metabolites can bind to the RNA during the extraction and form a insoluble complexes that hinder the isolation steps (Pawlowski *et al.*, 1994, cited in Liu *et al.*, 1998). Furthermore, others RNase contamination from glassware, skin and solutions also can susceptible to RNA degradation thus lowering the probability of success of RNA extraction (Rubio-Pina & Vazquez-Flota, 2008).

In this study, CTAB-LiCl method I, CTAB-LiCl method II, CTAB-LiCl method III and CTAB-isopropanol were used to extract RNA. For all of the method used, β-mercaptoethanol are added in the extraction buffer to prevent the oxidation of the samples and able to inhibits the RNase released from the tissues as a result of Chloroform extraction (Fu *et al.*, 2004). Previous research describde by Barlow *et al.*, (1963) has

proven that LiCl is effective in precipitating the RNA due to its ability that inefficiently precipitating the DNA, protein, and carbohydrates. The success in extraction of total RNA are determined by the high quantity and quality of RNA yet with less contamination of polysaccharides and degradation effect and finally able to achieve an intact bands of 28S and 18S rRNA,

The objectives of the study are:

1. To isolate RNA from pith of sago palm.
2. To study the effectiveness of methods for RNA extraction

2.0 LITERATURE REVIEW

2.1 *Metroxylon sagu*

Metroxylon sago which locally also known as sago palm has been described as humankind's oldest food plant by Avé (1977). Sago palm has a wide distribution all around the world started from Thailand, Peninsular Malaysia and Indonesia, to Micronesia, Fiji, and Samoa (McClatchey *et al.,* 2006). Sago palm was traditionally located in tropical lowland forest and freshwater swamps and usually found near the sea level. It also grown a long the shallow peat soil of river. The unique things about sago palm is that it can grow in wide variety of soil including well drained, poor quality sand and event in acidic peat swamp where mostly crops are unable to survive such acidic and extreme condition. The growth rate of sago palm is rapid where it can grow exceeding 1.5m according to 5 ft per year in an optimal condition. In term of size, it can reach up to 9 to 33m (30 to 108ft) (Figure 2.1) depending on what species it is. Sago palm has several function such coastal protection, decoration for home gardens, severe as commercial crops specially for starch production, animal food and fuel ethanol and until now sago palm still use as general consumption for rural villagers.

2.1.1 Lifecycle of *M.sagu*

Growth of sago palm is highly variable and depends on the condition of the soil. Sago palms propagate via sucker or via seed. Approximately, sago palm can grow up to 12 to18m in 6 to14 years. When the palms flower, the sucker formed. Then the sucker will grow further into rosette stage of leaves while the trunks are only formed after 4 to 6 years.

The growth of trunks can reached 6-14m. During that time, the trunk will show of 7 to 24 feathered leaves. A huge amount of inflorescence produced indicates the end of the life cycle. The formation of the inflorescence is where the starch produced in the trunk. The process starts at years 4 to 14. The suckers then will form and multiplies and form a cluster around the leader palm. There are certain names that are given by the local describing the growth of the sago palm. The most common names are described respectively according to stages, *Angkat punggung, Bibang, Pelawei, Pelawei manit, Bubul, Angau muda, Angau tua.* Meanwhile according to Flach's (1997) model (Figure 2.2), sago palm are divided into four stages during its life cycle and has 11-12 years of life cycle from seed to seed under the optimum ecological condition. The stages are, Rosette stage of 45 months from seeding, Bole formation stage of 54 months, inflorescence stage of 12 months and fruit ripening.

Figure 2.1: Morphology of *M.sagu.* Height of sago palm can reach up to 30-108ft.
(Source: http://www.sciencedirect.com/science/article/pii/S0144861707003852)

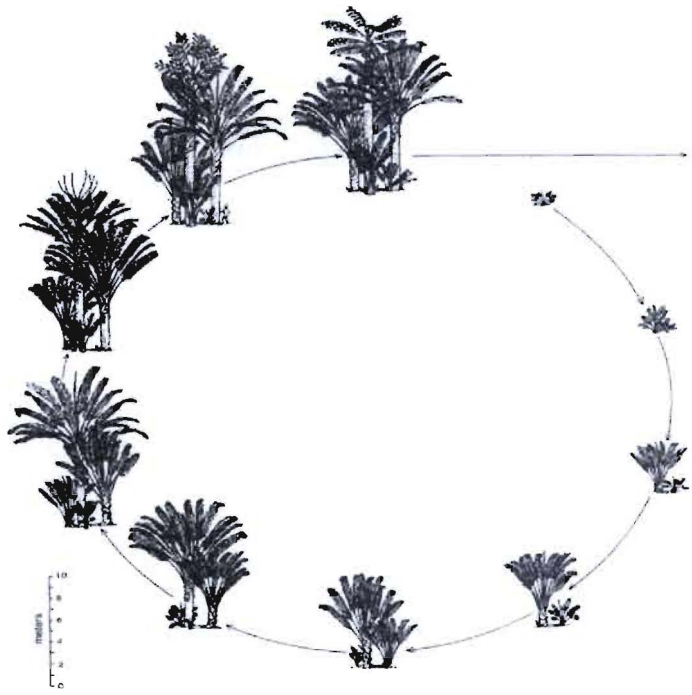

Figure 2.2 lifecycle of *M.sago*. Sago palm can grow up to 12 to 18m in 6 to 14 years. (Source: Schuiling and Flach, 1985)

2.1.2 Sago Pith

Sago pith is the inner part of the trunk, after the removal of the outer bark-like layer where the sago starch accumulates. Typically, the pith contains about 250kg of starch, 425kg water and 175kg of other material (Flach, 1983). The sago starch accumulates from the base upwards of the stem. At the maturity state, the trunk is fully saturated with starch and almost to the crown (Lang, Mohamed, & Karim, 2006). The starch productions were increase as the sago palm mature from *pelawei* to *angau muda* stage. However, it decreases as the sago palm at stage of *angau tua* to late *angau tua.* Besides, the sago pith also contains other related components. Based on statement described by Cecil *et al.* (1982) the chemical analysis of sago pith showed about 6 to 12% of soluble solids (dry substance),1

to 3% of ash and 79 to 88% of apparent starch with sugar. The sago pith also contained most of the constituents in any other plant materials name as fibre, hemicelluloses, other cell structural materials, soluble solids and some unidentified traces of other substances.

2.2 RNA Isolation

RNA isolation is a method use biotechnology to extract RNA from the taking sample. The samples can be in the form of animal tissues, plant material or even bacterial cell. The RNA isolation is for cDNA library construction. The cDNA library then will serve as the template for screening for genes which might be useful for the sago palm improvement later in the future. The RNA isolation method is slightly difficult when compared to DNA. This is because RNA is less stable than DNA, furthermore RNA are easily degraded than DNA. The example of established method of RNA isolation is using guanidine thiocyanate or hot acid phenol where guanidine thiocyanate are used as the protein denaturant or commercial TRIzol (Invitrogen, Carlsbad, CA, USA for direct cell lysis). However, this example is specialize for animal tissues rich in RNase but inappropriate for isolating high-quality RNA for other organisms. A lot of studies had been arise to overcomes this problem, for example a study conducted by Daldoul *et al.*, (2009) whereby constantly yielded high quality total RNA from grapevine. Crude RNA pellets were dissolved in borate-containing buffer, instead of normally used water before a selective lithium chloride precipitation, these step was found to be a critical step, leading to a 2.5 to fold increase of yield. Another example is a study conducted by Salzman *et al.*.(1999) of an Improved RNA Isolation Method for Plant Tissues Containing High Levels of Phenolic Compounds or Carbohydrates. The study explained on the difficulties of extracting high-quality RNA from inflexible plant tissues which are normally due to high levels of phenolics,

carbohydrates, or other compounds that bind and with RNA. In this study they use soluble polyvinylpyrrolidone (PVP) and ethanol precipitation. Using this method, RNA isolated from ripening grape berries, dry seeds of Albizia procera and radish, and leaf tissue of *A. procera* and *Griffonia simplicifolia* now capable to undergo PCR amplification and cDNA library construction. This method is also applicable to a variety of plant tissues.

2.3 RNA Quantification

RNA quantification is an important and necessary step prior to most RNA analysis methods. The traditional method for assessing RNA concentration and purity is UV spectroscopy. The absorbance of a diluted RNA sample is measured at 260 and 280 nm. A A260/A280 ratio of 2.0 is generally accepted as indicative of highly purified RNA. 230nm is use to check the contamination due to the solvent for example, phenol or ethanol. While, 280nm is indicate the contamination caused by protein.

3.0 MATERIALS AND METHODS

3.1 Sample Collection

For this study, pith from the bottom part of the palm was taken as a sample. The samples were obtained from Dalat area of Sarawak. Before the extraction can be done, the samples were first cut into thin slices and weighed approximately 1g and placed into different falcon tube. The falcon tube then stored at -20°C. While the extra and unprepared samples was stored at -80°C.

3.2. Materials

3.2.1 Plastic ware

The plastic ware that are going to be used for this study would be treated or rinse first using 0.1% DEPC for the purpose of deactivation of RNase activity that might contain on the plastic ware due to the fingermarks. After the treatment, the plastic ware will be sealed inside plastic bags and autoclaved at 120°C for 1 hour 30 minutes.

3.2.2 Pipette Tips

Especially for RNA work, disposable pipette tips were use along this study. The reasons are because the disposable pipette tips are normally RNase-free. DEPC treatments for pipettes are not needed but need to be autoclaved at 120°C for 1 hour 30 minutes.

3.2.3 Mortar and Pestle

Mortar and pestle were used in this study to grind the sample (pith) into powdery form, before it can be added into the extraction buffer. A reason for this step is to enhance extraction. Before use, mortar and pestle have to be autoclave at 120°C for 1 hour and 30 minutes. After that, the mortar and pestle were baked at 120°C overnight.

3.2.4 Solutions

The solution and water were treated with 0.1 % DEPC at 37°C overnight excluded for the buffer containing Tris. After that, the solution will undergo autoclaved at 120°C for 1 hour 30 minutes. For Tris buffer preparation, the water to be used was first treated with DEPC.

- *Extraction Buffer:*

2% CTAB, 2% PVP, 100mM Tris-HCl (pH 8.0), 25mM EDTA, 2.0M NaCl and 2 β-mercaptoethanol.

- *DEPC treated water:*

Distilled water was treated with diethyl-pyrocarbonate (DEPC) for overnight. After that, DEPC treated distilled water was autoclaved twice at 140°C for 1hour and 30 minutes. This step allowed the deactivation of the DEPC (Refer appendix).

3.3 RNA Isolation

This study comprise four methods of RNA isolation named as CTAB-LiCl method I, CTAB-LiCl method II, CTAB-LiCl method III and CTAB-isopropanol.

3.3.1 CTAB-LiCl Method I

This method was performed based on method of Gasic *et al.* (2004). All works were done in fume hood and on ice. Upon the grinding process, approximately 10ml of extraction buffer were place inside 50ml falcon tube and was pre-warmed in water bath at 60°C. 1g of sample were weighed and grind using mortar and pestle in assist of liquid nitrogen until powdery form of sample were produced. The sample then placed inside extraction buffer that has been pre-warmed. The mixture were subjected to vortex for 2 to 3 minutes and allowed to incubate in water bath of 60°C for 30 minutes. After 30 minutes of incubation, an equal volume of chloroform-isoamyl-alcohol (CIA) was added into the mixture. The

mixture then vortexed for 2 to 3 minutes. The mixture then was centrifuged at 8,000 rpm for 1 hour at 4°C.

After 1 hour of centrifugation, the supernatant was transferred approximately 700ml into each of 1.5 ml microcentrifuge tube. After that, an equal volume of CIA was added once more into the microcentrifuge tube. The mixture then was centrifuge at 13,000 rpm for 15 minutes at 4°C. After the centrifugation, the supernatant was transferred again into new 1.5 ml microcentrifuge tube. Then ¼ volume of 10M LiCl was cautiously added into each tube. The tubes were mixed by inversion and stored for overnight at -20°C.

After the overnight incubation, the samples are allowed to thaw on ice before it can be centrifuge at 13,000 rpm for 15 minutes at 4°C. After the centrifugation, the supernatant then discarded. The pellet then washed using 70% ethanol and was centrifuged at 13,000 rpm for 15 minutes at 4°C. After that, the supernatant was discarded and the pellets were allowed to air dry for 30 minutes. After 30 minutes, the pellets in each tube then dissolved in 35 µl of DEPC-treated distilled water. Once the pellets were dissolved, the RNA then was pooled into one 1.5 ml microcentrifuge tube. The pooled RNA then stored in -80°C for further utilize.

3.3.2 CTAB-LiCl Method II

In this method, approximately 1g of sample was ground using mortar and pestle with liquid nitrogen. Immediately after that, the sample was placed into 10 ml of CTAB buffer. The mixture of sample and buffer then incubate in water bath of 65^{0}C for 30 minutes. Straight after the incubation, the mixture then subjected to incubate on ice for 10 minutes. After that, half volume of Chloroform was added into the mixture. Then, the mixture was

subjected to centrifugation at 8000rpm for 30 minutes at 3°C. The supernatant then transfer into new falcon tube and half volume of LiCl were added into the solution. The mixture then allowed precipitating for 4 hours in -20°C. After 4 hours, the supernatant of the mixture then transfer into microcentrifuge tube. The tubes contain supernatant then centrifuge at 14, 000 rpm for 30 minutes at 4°C. After the centrifugation, the supernatant was discarded and the tubes were allowed to air dry. Finally the pellet were dissolved in DEPC-treated water and stored at -80°C for further use.

3.3.3 CTAB-LiCl Method III

Before the samples were ground into powdery form using liquid nitrogen, the extraction buffer were prewarmed at temperature of 65°C.Then, approximately 1g of sample were grind in liquid nitrogen and form powdery product. The powder state of sample then placed immediately after the grinding process into the falcon tube contained prewarmed extraction buffer. The mixture then allowed to incubate at temperature of 65°C for 10 minutes. An equal volume of CIA then added into the mixture and shake vigorously. The samples then subjected to centrifugation at 13000rpm for 10 minutes at 4°C. The supernatant resulted from the centrifugation then carefully transfer into new falcon tube. An equal volume of CIA once again added into the supernatant transferred and followed by centrifugation as above. After that, the supernatant were collected and placed into new tube. The supernatant then centrifuged at 15000rpm for 20 minutes at temperature of 4°C. The supernatant once again extracted and added with 0.25vol of LiCl. The mixture then allowed to mixed well and left overnight for precipitation. To recover the RNA, the sample then centrifuge at 15000rpm for 45 minutes at 4°C. After that, the supernatant was discarded and the pellets were washed using 70% ethanol 3 times. The tubes then allowed air dry and then dissolving in DEPC water ready for further analysis.

3.3.4 CTAB-isopropanol

Upon the grinding of sample, the extraction buffer was pre-warmed in water bath at 65°C. The powdered sample and 500µl of extraction were mixed together. 250µl of CIA was added into the mixture and vortexed to mix well the mixture. The mixture was then subjected to centrifugation at 13 000 rpm for 10 minutes at 4°C. After that, the supernatant was transferred into a new tube. Then, another 125µl of CIA was added into the mixture and vigorously shake to ensure the solutions were mixed well. Then, the mixture allowed to undergo process of centrifugation at 13 000rpm for 10mins at 4°C. After the centrifugation, the supernatant then transfer to new tube and 2 volume of Isopropanol were added into tube contain supernatant. The mixtures then incubate on ice for 5 minutes. The same step was repeated one more times. The supernatant then discarded and the pellet was washed using70% ethanol and stored at -20°C for 20 minutes. After the incubation, the mixtures then centrifuge once again and the supernatant were discarded and allowed to air dried. The pellets then dissolved in 70µl TE buffer and stored in -80°C for further use.

3.4 RNA Quantification

This process were performed to check the ratio of the concentration of RNA extracted where ratio of A260/280 and A260/230 are taken. 230nm indicate the present of solvent as contaminants. Examples of solvent are such ethanol isopropanol. Meanwhile 280 indicate the protein contamination. The expected ratio of a good RNA is in the range of 1.8-2.0. 10µl of samples were taken and mix with 990µl of DEPC water and the placed inside spectrophotometer to obtained readings of RNA yields.

3.5 RNA Analysis using Agarose Gel Electrophoresis

After the RNA are being extracted, the next step would be the electrophoresis step. This step is crucial to verify the size and integrity of RNA obtained. The apparatus for making gel were first set up then 0.3g of agarose was weighed and dissolved in 30ml of 1 x tris-acetate (TAE) buffer in a conical flask. After that, the solution will subjected to boil in a microwave for 3 minutes under medium heat. The heated solution then allowed to cool down to temperature range 55°C to 65°C. Then, 1µl of Ethidium Bromide (EtBr) was added for staining function. The mixture then swirled. The solution then poured into the set up apparatus comprises suitable tray and comb. The agarose gel then left for 30 minutes to solidify. After that, the harden gel then placed into electrophoresis tank and submerged by adding adequate TAE buffer. 5µl of sample then mixed with 1µl of loading dye (bromophenol blue) on the parafilm using pipette. The mixed samples were then carefully load into the well. The ladder (1kb and 100bp) also load into the well. The gel was then run for 30 minutes at 100 V. After the gel electrophoresis had been achieved, the gel brought to visualization under UV transilluminator.

4.0 RESULT AND DISCUSSION

4.1 Sample preparation

Bottom part of pith of sago palm was used as a sample and approximately 1g of samples was used for each the RNA isolation. The samples were obtained from the Dalat, Sarawak. The weight of the sample (lg) selected was an ideal weighed for this study because if the sample is less then 1g, the RNA isolated might be less and inadequate. Sago pith then cut into thin and tiny slices and weighed approximately 1g and placed in clean a 50ml falcon tube. The prepared samples were then stored at -20°C. However, this is not a proper way of storing samples, especially samples for RNA isolation as RNA is less stable and easy to degrade. Thus, it is better that to store the sample at -80°C, the samples are more stable and thus be able to preserve RNA better yet keep it longer for long terms use.

4.2 RNA Quantification

According to Rapley & Heptinstall (1998) the RNA have an ability to absorb approximately 260nm and thus the quality and quantity of RNA isolated can be verify spectrophometrically at 280nm and 230nm. 280nm indicate for protein contamination while 230nm indicate for polysaccharides contamination. Study conducted by Liu *et al.*, (1998) state that $A_{260/280}$ should have range of ratio from 1.8 to 2.0. The ratio less than 1.8 indicate the occurrence of protein contamination. In addition, $A_{260/230}$ should be greater than 1.8, any ratio below that indicates contamination by polysaccharides compound (Sangha *et al.*, 2010).

10µl of sample was diluted with 990µl of distilled water and subjected to spectrophotometer to identify the total yield of RNA isolated (Table 4.1). Based on result

obtained, 0.078 to 0.130 µg/µl of RNA yield are isolated using CTAB-LiCl Method I. At $A_{260/280}$, the ratio ranged from 1.004 to 1.703 while at $A_{260/230}$ the ratio was ranged from 0.540 to 0.644. For CTAB-LiCl Method II the ratio at $A_{260/280}$ was ranged from 1.260 to1.926 whereas at $A_{260/230}$ ratio was ranged from 0.400 to 0.493. Total yield of RNA from this method is ranged from 0.055 to 0.153 µg/µl. In other hand, CTAB-LiCl Method III has yielded total RNA of 0.025 to 0.144 µg/µl. At $A_{260/280}$ the ratio was ranged from 1.521 to 1.651 while at $A_{260/230}$ the ratio ranged from 0.534 to 1.550. In contrast, CTAB-Isopropanol has yielded total RNA of 0.050 to 0.065 µg/µl. At $A_{260/280}$ the ratio ranged from 1.518 to 1.707 and at $A_{260/230}$ the ratio ranged 0.500 to 0.603. From this reading of ratio and total yield of RNA, the ratio less than 1.8 indicating poor quality of RNA isolated.

Table 4.1: Spectrophotometric analysis for total RNA absorbance and total RNA yields.

Method	Sample	A_{230}	A_{260}	A_{280}	$A_{260/280}$	$A_{260/230}$	RNA yield (µg/µl)
CTAB-LiCl Method I	1	0.204	0.287	0.181	1.486	0.540	0.078
	2	0.093	0.077	0.077	1.004	0.644	0.116
	3	0.226	0.203	0.190	1.730	0.589	0.130
CTAB-LiCl Method II	1	0.063	0.042	0.039	1.260	0.400	0.055
	2	0.202	0.163	0.144	1.926	0.493	0.153
CTAB-LiCl Method III	1	0.127	0.121	0.119	1.602	0.534	0.025
	2	0.075	0.088	0.074	1.651	1.550	0.144
	3	0.220	0.218	0.206	1.521	0.930	0.135
CTAB-Isopropanol	1	0.244	0.232	0.227	1.707	0.500	0.050
	2	0.247	0.236	0.231	1.518	0.603	0.065

The result of spectrophometer analysis also shown that CTAB-LiCl Method III is somewhat free from contamination if compare to method CTAB-Isopropanol with ratio at A260/230 ranged from 0.500 to 0.603, which indicating high contamination of polysaccharides compound. The result also proved that CTAB-LiCl method is more efficient than CTAB-isopropanol in isolating RNA molecule. The reason for the effectiveness of CTAB-LiCl method is because LiCl is ineffective in precipitating other compounds such as DNA. Furthermore, it allow more accurate reading when check with UV spectrophotometer.

4.3 Total RNA Isolation

In this study, the RNA was isolated from bottom part of pith and continued with the analysis of the total RNA via electrophoresis and RNA quantification method in order to verify the quantity and quality of RNA.

4.3.1 CTAB-LiCl Method I

1g of powdery form of sago pith was used to isolate total RNA. The bands were examined on the gel with slightly smearing along the lanes (Figure 4.1). The smearing indicates that the RNA was contaminated yet might be degraded. The electrophoresis result for this CTAB-LiCl Method I was similar to the gel analysis of total RNA isolated by Singh's *et al*, (2003). Smearing that indicates the contamination might due to the RNase contamination via glassware and via solution. The RNA might also be degrade when incorrect way of grinding in liquid nitrogen. Excess of use of liquid nitrogen also can cause the RNA to degrade.